soluble herbage proteins are associated with the occurrence of bloat in cattle.

ACKNOWLEDGMENT

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Changes with Maturity in Anatomy, Histochemistry, Chemistry, and Tissue Digestibility of Bermudagrass Plant Parts

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Coastal bermudagrass (*Cynodon dactylon* (L.) Pers.) was harvested after 5.5 months of greenhouse regrowth and separated into leaf blades, sheaths, and stems of top, middle, and bottom portions to reflect differences in maturity of each plant part. The parts were evaluated for differences associated with maturity in the percentages of tissue types, histochemistry of sections, and compositional analyses, and these data were related to changes in the in vitro dry matter digestibility (IVDMD) and in the degradation of specific tissue types observed by scanning electron microscopy. In blades, no significant differences with maturity (as related to digestibility) were found among the parameters. In sheaths and stems, acid detergent fiber (ADF) and lignin increased with maturity. Histochemical studies indicated the presence of chlorine sulfite positive lignin in the mesophyll of sheaths and parenchyma of stems of middle and bottom portions only. These tissues were less degraded in the bottom sheath and stem portions, and their IVDMD decreased with maturity. Decreases in digestibility with maturity of bermudagrass appeared to be at least partially the result of chlorine sulfite positive lignin in thin-walled cells of bottom portions of sheaths and stems.

The effects of maturity on forage grasses have been studied on the basis of several criteria: chemical composition (Burton et al., 1964; Danley and Vetter, 1973; Deinum and Dirven, 1971; Moore et al., 1970), morphology (Johnston and Waite, 1965; Prine and Burton, 1956), and animal performance (Utley et al., 1971; Weston and Hogan, 1968). Additionally, decreases in forage digestibility with maturity have been shown in both warm-season grasses (Danley and Vetter, 1973; Burton et al., 1964; Moore et al., 1970; Ventura et al., 1975; Wilkinson et al., 1970) and cool-season grasses (Mowat et al., 1965; Pritchard et al., 1963; Terry and Tilley, 1964). More specifically, Coastal bermudagrass, pelleted at 4 and 8 weeks of age, showed a decrease in dry matter digestibility of about 10 percentage units in the older vs. the younger forage (Utley et al., 1971). Wilkinson et al. (1970) reported that the bottom portion of Coastal bermudagrass was 17 to 20 percentage units lower in dry matter digestibility than the top portion.

Reported decreases in the digestibility among the plant parts due to maturity have been consistent. Leaf blades usually decrease in digestibility, but not as much nor as rapidly as the stems (Pritchard et al., 1963; Mowat et al.,

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1965). Leaf sheaths were intermediate between blades and stems in the decrease in digestibility with maturity of cool-season grasses (Terry and Tilley, 1964).

Histochemical studies have been used to show changes with maturity in cool-season grasses (Pigden, 1953; Johnston and Waite, 1965; Wilkins, 1972), clover (Drapala et al., 1947), and recently in stems of a warm-season grass (Schank et al., 1973). While not used heretofore to study changes in tissue digestibility due to maturity, the scanning electron microscope (SEM) has been useful in investigating the relative ease of digestibility of tissue types in various forages (Akin et al., 1973).

The objectives of this study were to compare leaf blades, sheaths, and stems divided into top, middle, and bottom portions grown to maximize factors occurring with maturity in Coastal bermudagrass. The grass was grown under constant greenhouse conditions to reduce influences of environmental variations such as temperature, rainfall, or light intensity. Plant parts were compared for differences in the amounts of tissue types (top and bottom portions only), in histochemistry, and in chemical constituents as related to decreases in tissue digestibility using the SEM and to differences in in vitro dry matter digestibility (IVDMD).

EXPERIMENTAL SECTION

Preparation of Grass Samples. Coastal bermudagrass (*Cynodon dactylon* (L.) Pers.) was grown in a greenhouse in three galvanized metal flats (100 × 70 × 20 cm) with 6.4 mm holes on 15.2 cm centers in the bottom for drainage. The grass was cut 2 cm from the ground and grown for 5.5 months with a fertilization rate of 28 kg of N, 9.4 kg of P_2O_5, and 19.7 kg of K_2O per ha every 2 weeks and watered to saturation every second day. The greenhouse temperature ranged from about 24 to 32 °C, and relative humidity averaged 60%. The plants were exposed to sunlight through clear glass, received no supplemental lighting, and were grown from February to July.

After 5.5 months regrowth, plants were harvested 2 cm from the soil surface and separated into a young portion (top 3 to 4 blades with associated sheath and stem), an old portion (bottom 2 to 4 green leaves with associated sheath and stem), and a middle portion removed halfway between the top and bottom of each plant. Each portion was then separated into leaf blades, sheaths, and stems. Samples were quick-frozen in dry ice and stored at −30 °C until used.

Compositional Analysis. A portion of each sample was freeze-dried for chemical analyses. Crude protein was determined by the Kjeldahl method (AOAC, 1960), acid detergent fiber (ADF) by the method of Van Soest (1963), and neutral detergent fiber (NDF) and permanganate lignin (PML) by the methods of Van Soest and Wine (1967, 1968, respectively). Hemicellulose was determined as the difference in NDF minus ADF. Cellulose was determined by loss in dry matter upon ashing the residue from the PML determination. Glassware for Van Soest analyses was modified as described (Akin et al., 1975).

Anatomical and Histochemical Determinations. Fresh-frozen top and bottom portions were embedded in the medium of Wills and Briscoe (1970) and frozen in a cryostat. Sections 16-μm thick were cut and stained with acid phloroglucinol or chlorine sulfite (Jensen, 1962) used previously to indicate different types of lignins (Stafford, 1962). Duplicate slides of 8 to 10 sections of each plant part were stained with each of the lignin stains and micrographs at a total magnification of ×50 were made into 2 × 2 projector slides.

For percentages of tissue types, the slides were projected onto graph paper and the squares under similar tissues counted and calculated as a percentage of the total observable area at 10× objective lens magnification. Stems were round, not linear as blades and sheaths. Therefore, since only a portion of the stem was observed at 10× objective lens magnification, a modified counting method was used to ensure determination of an equal proportion of tissues on the inner and outer regions of the stem. Slides of stem portions were projected onto graph paper as with blades and sheaths. The approximate center of the stem was found by dropping two perpendicular lines from the tangent of the arc of the stem periphery and extending the perpendicular lines until they intersected on the graph paper. A protractor was placed at this intersection and angles, usually 25 to 60°, were drawn to include the area observable in the slide. Tissue types were counted as above within this "pie-slice" area.

Digestibility Determinations. Duplicate runs for IVDMD were made in triplicate on each portion by the procedure of Tilley and Terry (1963). The relative rates and types of tissues digested were determined by SEM examination of plant portions degraded in vitro by rumen microbes. Sections 5-mm long of each plant part were cut so that regions near the ends of the blades and sheaths and the nodes of the stems were avoided. About 30 sections total were cut from 10 to 15 of each of the blades, sheaths, and stems placed in 50-ml centrifuge tubes. Rumen fluid from a cannulated steer was double-strained through multi-layered cheesecloth, mixed with 2 parts of McDougall's carbonate buffer (1948), and added to tubes in equal amounts by an automatic dispensor. The tubes were then incubated with continuous CO_2 bubbling at 39 °C. Samples were removed after 5, 11, 24, 48, and 72 h of incubation. Control sections of top parts of the plant were removed after 72 h of incubation in buffer alone with CO_2 bubbling at 39 °C. The sections were prepared for the SEM and the transmission electron microscope (TEM) as described (Akin et al., 1975; Akin et al., 1974, respectively) except that samples for the SEM were critical point dried in liquid CO_2 rather than freeze-dried. The experiment was repeated on top and bottom plant parts for 6, 24, and 72 h of incubation with controls as above, but with 250 ml of inoculum in 500-ml flasks for all samples.

RESULTS

The percentages of tissue types for top and bottom parts are shown in Table I. In blades, most tissues did not change, but the amount of parenchyma bundle sheath tissue increased with maturity resulting in an increase in total vascular tissue and decrease in mesophyll; these differences were significant at the 0.05 level, but not at the 0.01 level. Lignified tissues indicated by a positive acid phloroglucinol reaction (P^+) were found only in the xylem cells and inner bundle sheath of large vascular bundles (Figure 1, X and I). The sclerenchyma stained positive for lignin with the chlorine sulfite stain (CS^+) (Figure 1, S). Neither percentage nor sites of lignified tissue changed with maturity.

The sheaths, which are botanically part of the leaf, differed with maturity only in the amount of the sclerenchyma-like tissue on the adaxial side (significant at the 0.05 but not at the 0.01 level) and a slight difference in phloem (Table I). P^+ lignin was found in a ring of cells surrounding vascular tissue analogous to the inner bundle sheath of the blade (Figure 2, I); slight P^+ staining occurred in the sclerenchyma cells between vascular bundles and epidermis (Figure 2, S). This sclerenchyma tissue was CS^+ also, and CS^+ lignin appeared in the sclerenchyma-like

Table I. Percent Tissue Types in Top and Bottom Portions of Plant Parts in Coastal Bermudagrass

Tissue	Top	Bottom
	Blades	
Mesophyll	37.6 ± 4.4[a]	28.9 ± 8.7
Epidermis	20.8 ± 4.3	22.6 ± 5.2
Sclerenchyma	13.5 ± 2.7	15.5 ± 3.1
Total vascular tissue	28.2 ± 3.7	33.0 ± 5.3
Lignified vascular tissue	6.2 ± 1.6	6.1 ± 1.5
Parenchyma bundle sheath	17.2 ± 3.0	22.5 ± 4.8
Phloem	4.8 ± 6.0	4.4 ± 1.1
	Sheaths	
Mesophyll	59.2 ± 8.5[a]	59.8 ± 4.5
Sclerenchyma (adaxial)	12.9 ± 3.1	10.3 ± 1.6
Lignified vascular bundle and sclerenchyma (adaxial)	17.0 ± 5.1	19.5 ± 2.4
Parenchyma bundle sheath	7.4 ± 2.3	8.6 ± 1.9
Phloem	2.1 ± 0.4	1.8 ± 0.4
	Stems	
Parenchyma	56.3 ± 5.2[a]	60.0 ± 5.7
Lignified band (sclerenchyma, vascular bundles, and epidermis)	39.4 ± 5.3	36.7 ± 5.5
Phloem	4.4 ± 1.9	3.4 ± 0.7

[a] Average determinations from eight to ten samples and standard deviation.

Table II. Percent Compositional Analysis in Top, Middle, and Bottom Portions of Plant Parts in Coastal Bermudagrass

Constituents	Top	Middle	Bottom
		Blades	
Crude protein	13.7 ± 0.1[a]	11.9 ± 0.0	10.7 ± 0.0
Neutral detergent fiber	72.1 ± 1.7	70.3 ± 0.7	66.6 ± 1.0
Acid detergent fiber	31.5 ± 0.6	30.6 ± 0.4	30.6 ± 0.5
Permanganate lignin	2.7 ± 0.1	2.6 ± 0.1	2.8 ± 0.1
Hemicellulose	40.6[b]	39.7	36.0
Cellulose	28.8 ± 0.1	27.4 ± 0.6	27.6 ± 0.8
		Sheaths	
Crude protein	8.6 ± 0.0	5.9 ± 0.0	5.4 ± 0.1
Neutral detergent fiber	76.6 ± 0.4	79.0 ± 0.1	76.0 ± 0.2
Acid detergent fiber	36.5 ± 0.3	40.8 ± 1.2	40.2 ± 0.2
Permanganate lignin	5.0 ± 0.2	7.9 ± 0.9	8.5 ± 0.4
Hemicellulose	40.1[b]	38.2	35.8
Cellulose	30.9 ± 0.1	31.9 ± 0.4	30.6 ± 0.4
		Stems	
Crude protein	10.6 ± 0.4	3.2 ± 0.0	3.0 ± 0.1
Neutral detergent fiber	67.0 ± 0.3	72.6 ± 0.5	78.3 ± 1.1
Acid detergent fiber	33.7 ± 0.9	41.7 ± 2.5	43.6 ± 1.3
Permanganate lignin	4.6 ± 0.2	8.7 ± 0.5	7.9 ± 1.6
Hemicellulose	33.3[b]	30.9	34.7
Cellulose	29.9 ± 0.4	33.8 ± 1.8	35.7 ± 1.3

[a] Average of triplicate determinations and standard deviation. [b] Values were determined from percent NDF–ADF.

tissue on the adaxial side (Figure 2, C). Mesophyll cells (Figure 2, M) in the middle and bottom sheath portions occasionally showed some CS^+ lignin, whereas no reaction was seen in the top sheaths.

In stems, no significant differences due to maturity were found in the percentages of parenchyma cells (pith and cortex), the lignified sclerenchyma band plus vascular bundles, or the phloem inside the vascular bundles (Table I). The lignified sclerenchyma band plus vascular bundles (Figure 3, S, V) were P^+ and in top and bottom portions. The parenchyma cells did not stain positively for lignin in top stems, but were CS^+ (strongly at times) in both middle and bottom portions (Figure 3, C).

Compositional Analyses. Changes with maturity were found in the percentages of some of the cell wall constituents (Table II). Crude protein decreased from top to bottom in all plant parts, but the decrease was greatest in stems. Values for ADF and PML were similar in blades of all portions, but increased in sheaths and stems with maturity.

Table III. In Vitro Dry Matter Disappearance of Top, Middle, and Bottom Portions of Plant Parts in Coastal Bermudagrass

Plant part and maturity	Trial 1	Trial 2	Av
Top blade	53.0 ± 0.5[a]	54.0 ± 1.1	53.5
Middle blade	48.3 ± 0.2	52.9 ± 1.1	50.6
Bottom blade	52.4 ± 1.8	57.0 ± 1.2	54.7
Top sheath	55.8 ± 0.3	57.8 ± 1.1	56.8
Middle sheath	42.1 ± 0.7	45.8 ± 0.3	44.0
Bottom sheath	37.2 ± 1.0	49.8 ± 5.0	43.5
Top stem	59.9 ± 0.7	61.8 ± 0.5	61.0
Middle stem	41.4 ± 0.8	43.4 ± 0.4	42.4
Bottom stem	42.4 ± 0.7	48.0 ± 1.5	45.2

[a] Average of triplicate determinations and standard deviation.

Digestibility. IVDMD values are shown in Table III. Data in trials 1 and 2 agreed except for some bottom portions. The average values indicated that blades remained the same or perhaps increased slightly in digestibility, while both middle and bottom sheaths and stems decreased.

The SEM revealed the types of tissues present in the grass parts and showed that all cells were intact in sections incubated in control buffer for 72 h (Figures 4, 5, and 6). Large vacuoles, seen on occasion in control sheaths (Figure 5, Vc), were not considered to be degraded regions. In blades, tissues were removed equally from top and bottom portions at the selected times of incubation; mesophyll and phloem were completely degraded after 72 h (Figures 7 and 8). However, even after 72 h, parenchyma bundle sheath and epidermis remained showing only partial digestion; the lignified tissues were not degraded (Figures 7 and 8). Sclerenchyma tissue associated with vascular bundles was difficult to find after 72 h of incubation.

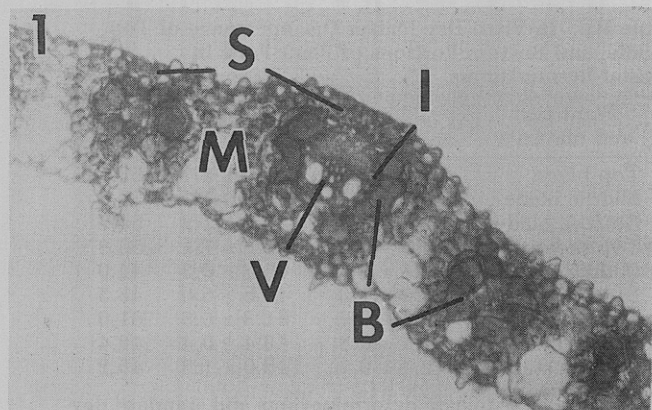
The bottom leaf sheaths were usually less degraded than the top sheath or the blades (Figures 9 and 10). Phloem was degraded early while the parenchyma bundle sheaths in both top and bottom portions were undegraded even after 72-h incubation (Figures 11 and 12). Mesophyll cells were not consistently degraded in all sections; the top sheath portions were degraded to a greater extent than bottom portions after 72 h. However, bottom sections were often degraded in the central portion of sheath (Figure 10) and at times some mesophyll remained in top sheaths (Figure 11).

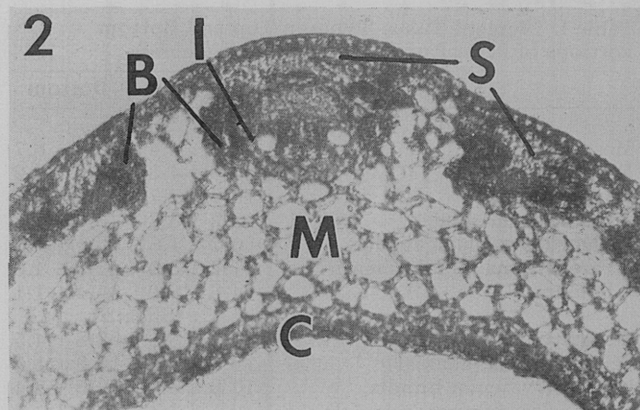
In stems, differences due to maturity were apparent even after only 6-h incubation; parenchyma cells were degraded in top (Figure 13) but remained intact in bottom portions (Figure 14). Phloem was degraded early while the lignified band, vascular bundles, and epidermis were intact at both maturities. After 72-h incubation, the P^+ regions remained in both top and bottom stems, but differences were apparent in the parenchyma (Figures 15 and 16), which was completely digested in top stems but only slightly digested in bottom stems. Middle portions more nearly fit the digestive pattern for bottom sections in sheath and stem portions.

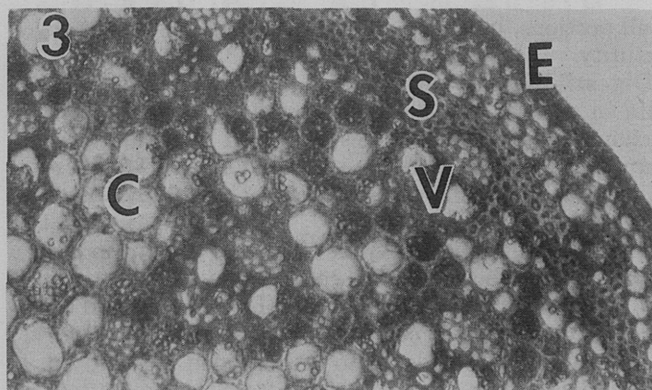
Transmission electron micrographs indicated that rumen bacteria attacked and degraded the parenchyma cells in top stems (Figure 17). Although some degradation occurred in bottom stems (Figure 18), many cell walls appeared to resist bacterial degradation.

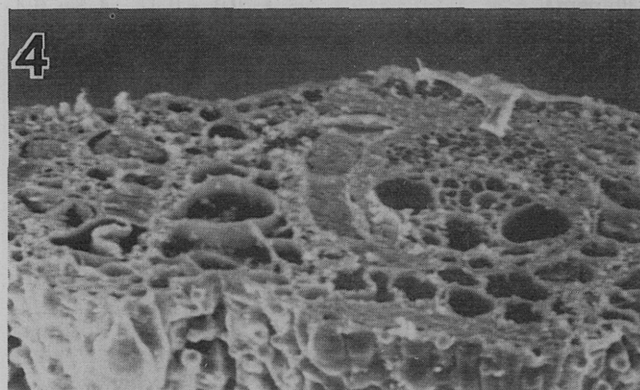
DISCUSSION

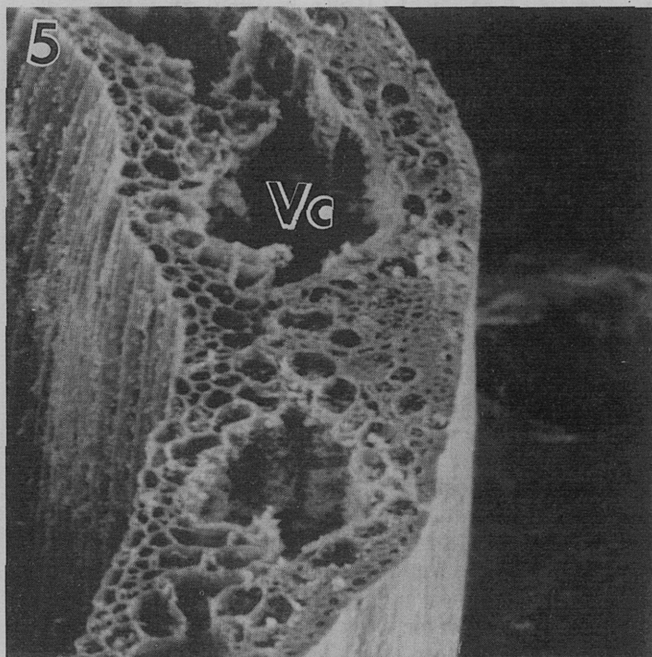
Histochemical, chemical, and digestibility data for different maturities of Coastal bermudagrass blades were











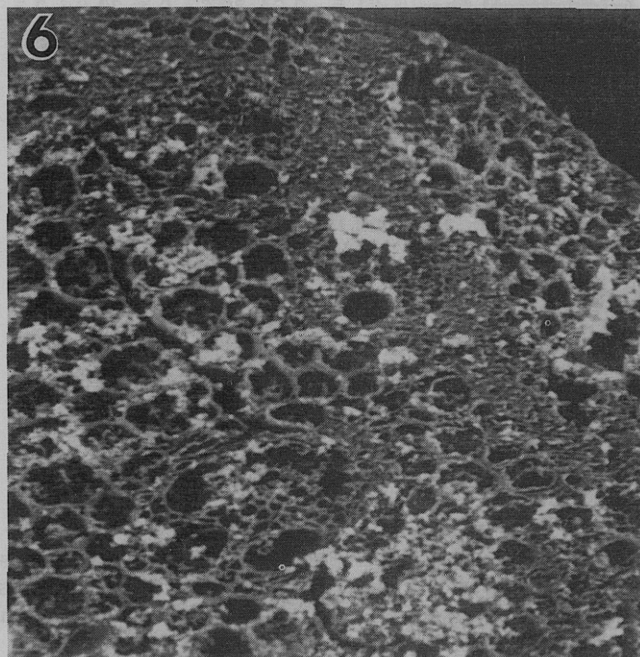


Figure 1. *Light micrograph of leaf blade. Lignified tissue stained* with acid phloroglucinol is in the inner bundle sheath (I) and xylem of the large vascular bundle (V) while the sclerenchyma (S) stains with chlorine sulfite. The parenchyma bundle sheaths (B) are large, unlignified cells surrounding the vascular bundles, which are separated by mesophyll (M); ×205.

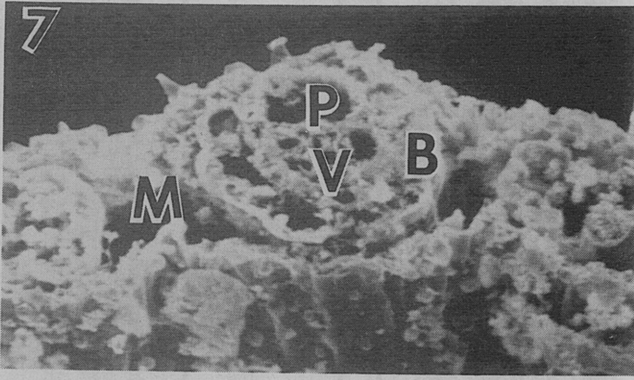
Figure 2. Light micrograph of leaf sheath. Lignified tissue shown by acid phloroglucinol is found mostly in a small layer (I) around phloem and xylem of the vascular bundles. The sclerenchyma (S) connecting the bundles to the epidermis stains slightly with acid phloroglucinol but more so with chlorine sulfite, while the sclerenchyma cells (C) at the adaxial side stains with chlorine sulfite. Parenchyma bundle sheaths (B) surround vascular bundles, and mesophyll (M) is between the epidermises; ×200.

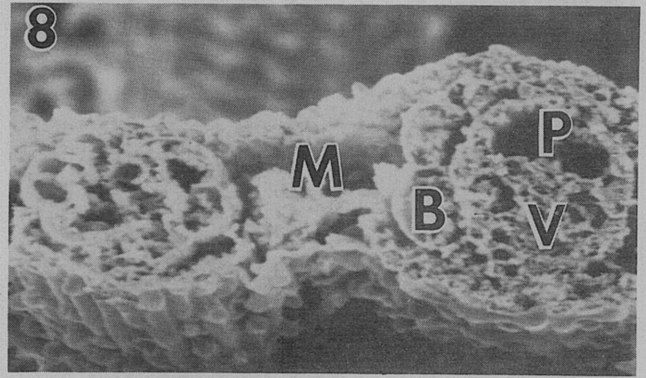
Figure 3. Light micrograph of stem. Lignified tissue in the epidermis (E) and in a thick band of sclerenchyma (S) and vascular bundles (V) (except phloem) stains positive with acid phloroglucinol. Parenchyma cells (C) stain positive for lignin with chlorine sulfite only in older sections; ×200.

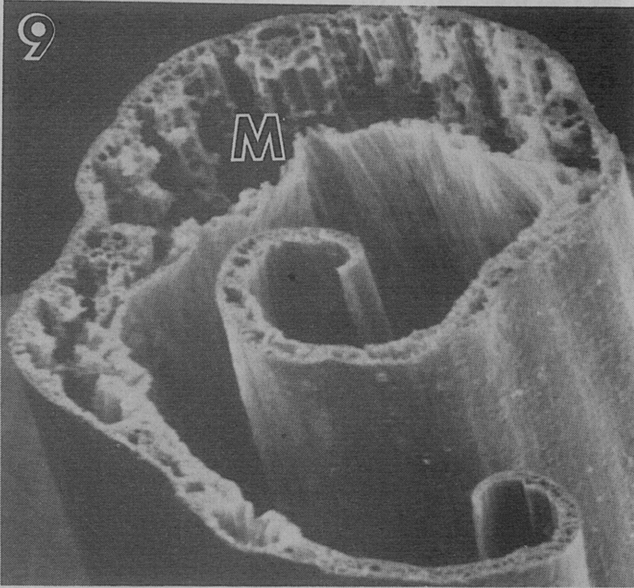
Figure 4. Control top blade section incubated in buffer for 72 h shown by SEM. All tissues are intact; ×352.

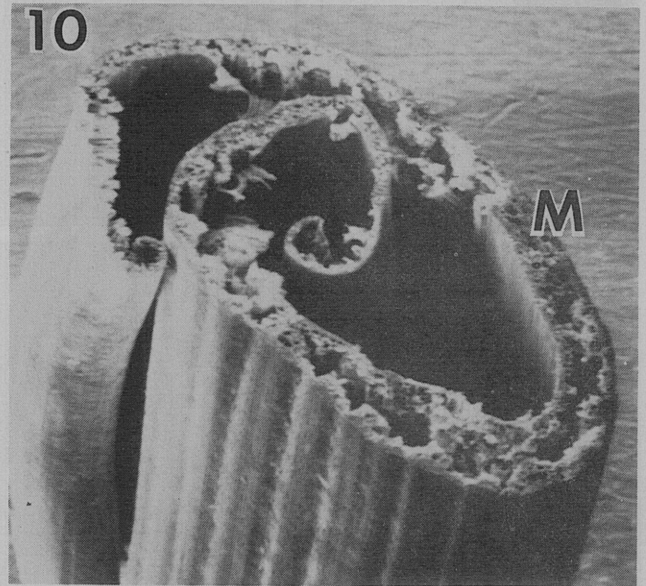
Figure 5. Control top sheath section incubated in buffer for 72 h shown by the SEM. Tissues are intact although vacuoles (Vc) are seen in the mesophyll region; ×352.

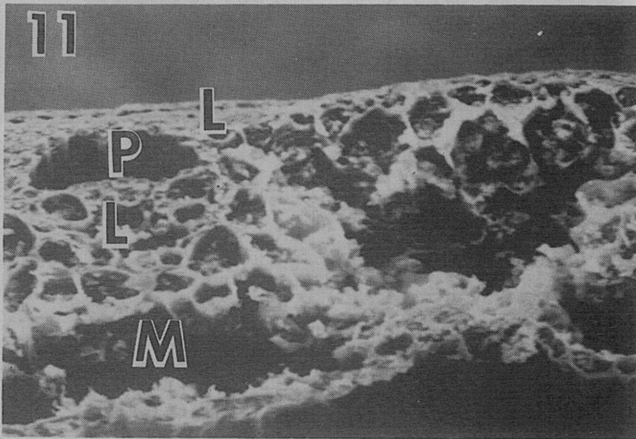
Figure 6. Control top stem section incubated in buffer for 72 h shown by the SEM. Tissues are intact showing no removal due to buffer; ×352.











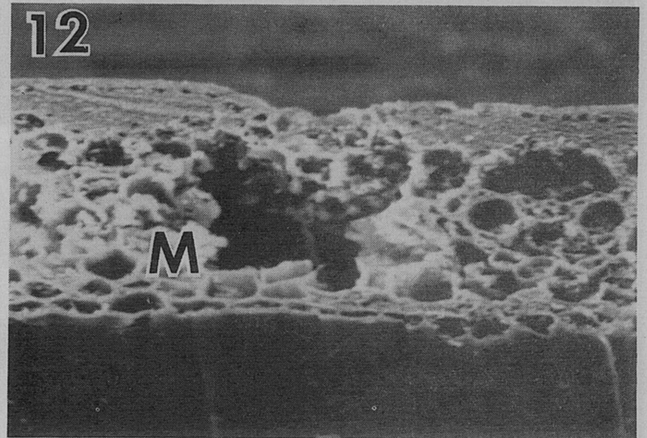


Figure 7. Top blade section digested for 72 h shown by SEM. Mesophyll (M) and phloem (P) are degraded while parenchyma bundle sheaths (B) are partially degraded and lignified vascular tissues (V) are intact; ×352.

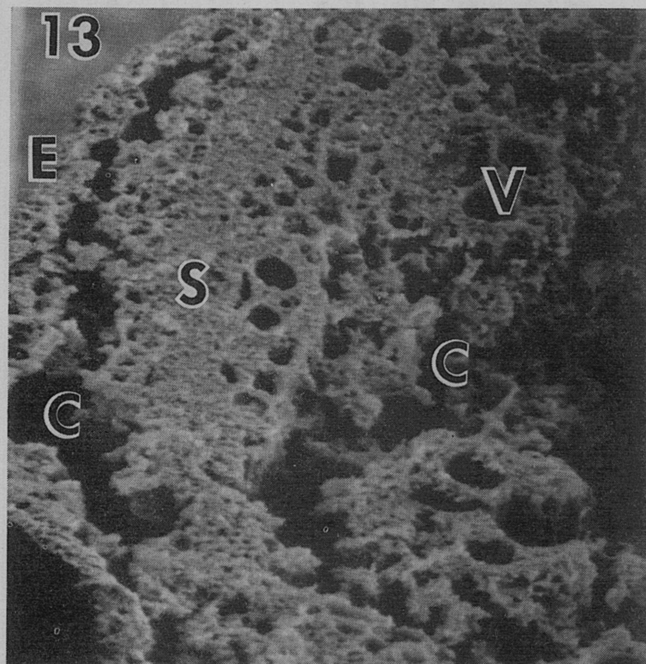
Figure 8. Bottom blade section digested for 72 h shown by SEM. Changes are the same as for top blade in mesophyll (M), phloem (P), parenchyma bundle sheath (B), and lignified vascular tissue (V); ×384.

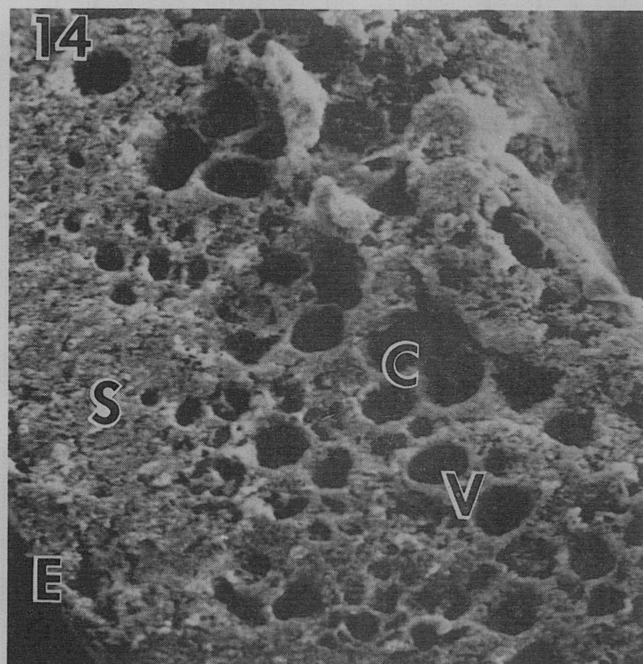
Figure 9. Top leaf sheath section digested for 72 h shown by SEM. Mesophyll cells (M) are degraded to a large extent; ×120.

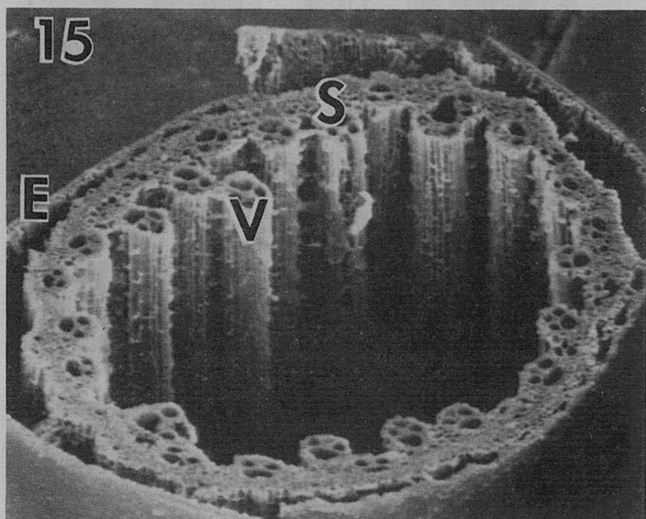
Figure 10. Bottom leaf sheath section digested for 72 h shown by SEM. More mesophyll (M) remains than in the top section; ×55.

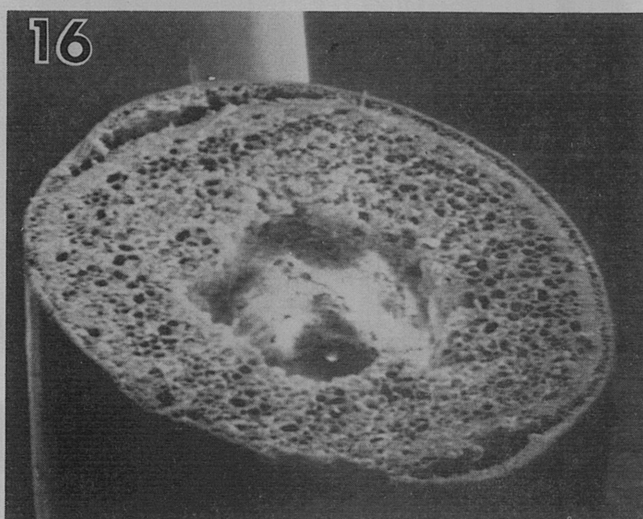
Figure 11. Enlargement of top leaf sheath digested 72 h shown by SEM. Phloem (P) and some mesophyll (M) are removed while the parenchyma bundle sheath (B) is partially degraded and lignified regions (L) are intact. Vacuoles are seen as in Figure 5; ×384.

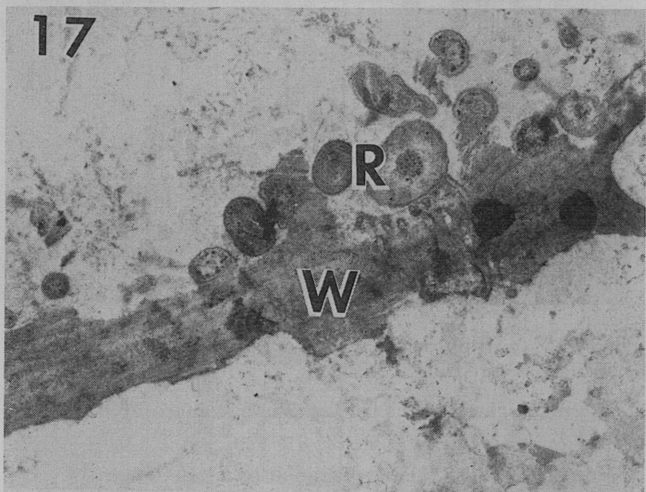
Figure 12. Enlargement of bottom leaf sheath section digested for 72 h shown by SEM. Less mesophyll (M) is removed than in the younger sheath sections. Phloem is removed while lignified tissues remain. Empty spaces analogous to vacuoles in Figure 5 are seen; ×352.











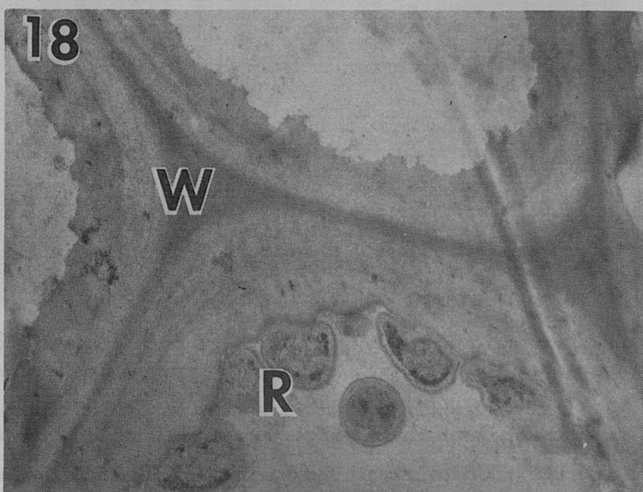


Figure 13. Top stem digested for 6 h shown by SEM. Parenchyma cells (C) are being degraded while lignified epidermis (E) and sclerenchyma band (S) with vascular tissues (V) remain; ×220.

Figure 14. Bottom stem digested for 6 h shown by SEM. Parenchyma cells (C), epidermis (E), sclerenchyma band (S) with vascular tissues (V) show no digestion; ×220.

Figure 15. Top stem digested for 72 h shown by SEM. The epidermis (E), sclerenchyma band (S), and vascular bundles (V) remain while the nonlignified parenchyma and phloem cells are degraded; ×69.

Figure 16. Bottom stem digested for 72 h shown by SEM. All tissues appear intact; ×55.

Figure 17. Cell wall (W) of top stem degraded by rumen bacteria (R) shown by TEM; ×9720.

Figure 18. Cell wall (W) of bottom stem only slightly degraded by attached rumen bacteria (R) shown by TEM. The study shows bacteria near completely undegraded cell walls; ×25 500.

consistent in that sites and amounts of lignin remained constant as did, in general, the digestibility of tissue types observed by the SEM and IVDMD. The amounts of total vascular and parenchyma bundle sheath tissue were lower than published accounts (Akin and Burdick, 1975) probably due to different counting techniques. Others have reported some decrease in digestibility with leaf maturity (Deinum and Dirven, 1971; Burton et al., 1964). However, the blade IVDMD of high- and low-quality bermudagrass genotypes was not found to change within cultivars from 1 to 4 weeks of age (Hanna et al., 1976). The decrease in IVDMD of middle blades might be attributed to sample variation, but the decrease occurred in both trials. Additionally, our samples did not contain dead leaves which are less digestible than live ones in some plants (Mowat et al., 1965). In previous work, parenchyma bundle sheaths and epidermis of field-grown Coastal bermudagrass (4 to 8 weeks old) were completely digested after 72 h (e.g., Figure 7 in Akin et al., 1973). In the present study of greenhouse samples these tissues were only beginning to be digested. Furthermore, the greenhouse samples were lower in IVDMD than field-grown samples at 4 weeks regrowth. The differences in tissue digestibility may be due to the extensive age (5.5 months) of the plants or to some condition in the greenhouse which made these thick-walled cells less subject to degradation. For example, in tall fescue Deinum and Dirven (1971) showed a decrease in digestibility not due to lignin with an increase in temperature. It did not appear that the differences associated with maturity in the amounts of tissues affected blade digestibility in our samples.

Little information is available on sheath anatomy in grasses. Although the sheath along with the blade comprises the leaf, we found differences in the tissue types between these components (Table I). Wilkins (1972) reported increases in the sclerenchyma of sheaths and blades that affected potential digestibility in cool season grasses, but in our study the main difference relating to digestibility in sheaths was in the mesophyll. In both middle and bottom sheaths, CS^+ lignin was found in the mesophyll with concomitant increases in ADF and PML. Furthermore, the mesophyll cells in some samples were resistant to digestion by rumen bacteria. These observations indicated that in older sheaths lignification in the mesophyll area could at least partially explain decreased digestibility. Terry and Tilley (1964) reported that the decrease in sheath digestibility was intermediate between that of the blades and stems in cool season species, but our IVDMD results indicated that decreased sheath digestibility more closely paralleled the stems of Coastal bermudagrass. However, tissue digestion shown by the SEM appeared greater in sheaths than stems after 72-h incubation (Figures 12 and 16).

Differences in histochemistry related to digestibility were most apparent in the stems. The amounts of tissue types did not differ with maturity, and the P^+ lignified regions (i.e., the epidermis, sclerenchyma band, and vascular bundles) were not different histochemically. These data did not confirm work by Johnston and Waite (1965) who found progressive P^+ lignification of the fibrous pericycle and suggested this might lower stem digestibility in some cool-season grasses. However, in mature stems parenchyma cells stained CS^+ and these cells were not degraded even after 72 h. This increase in stem lignification was confirmed chemically (Table II) and the decrease in digestibility by IVDMD (Table III). Pigden (1953) reported that with maturity heavy P^+ lignification occurred in parenchyma cells adjacent to vascular bundles in cool-season forage grasses. Johnston and Waite (1965) reported that some P^+ lignification occurred in stems of the more mature plants in the "larger cells which formed the connective tissue between the major vascular bundles". Our data agreed with those of other reports (Pritchard et al., 1963; Mowat et al., 1965; Deinum and Dirven, 1971; Terry and Tilley, 1964; Hanna et al., 1976) which showed a greater decrease in digestibility of stem vs. leaves with maturity.

In our study, changes with maturity in the patterns of NDF (cell walls), cellulose, and hemicellulose could not be associated with digestibility changes of plant parts. Decreases in crude protein with maturity had been shown for Coastal bermudagrass (Utley et al., 1971; Wilkinson et al., 1970) and in our study were greater in sheaths and stems than in blades. The changes in ADF and PML paralleled changes in digestibility in all plant parts, whereas histochemical data showed that the increases in lignin appeared to result from increased CS^+ lignin in mesophyll and parenchyma cells of sheaths and stems, respectively. Stafford (1962) showed that chlorine sulfite and acid phloroglucinol differentiated various kinds of lignins in ryegrass with syringyl-type lignin staining more readily with chlorine sulfite. The chlorine sulfite reagents became ineffective after a short time and gave false-negative reactions. It was necessary to prepare new solutions several times daily to obtain consistent results.

Kamstra et al. (1958) thought of lignin as a physical, encrusting barrier preventing the action of rumen microbial enzymes on cell walls. Morrison (1974) isolated lignin–carbohydrate complexes with dimethyl sulfoxide from ryegrass and postulated, from the similarity between the composition of the complex and intact cell wall, that these lignin–carbohydrate complexes could be units of the cell wall structure. Lai and Sarkanen (1971) reviewed efforts to isolate covalently bonded lignin–carbohydrate complexes and discussed possible linkages. They reported that circumstantial data suggested that covalent bonds between lignin and carbohydrates exist, but definitive evidence to date was lacking. Further studies on the association of different types of lignin with cell wall constituents should give additional insight to the factors which limit the degradation of cellulose and hemicellulose by rumen microorganisms.

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Potential Sources of Peanut Seed Proteins and Oil in the Genus *Arachis*

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Fat-free and full-fat seed meals from 37 selected wild species had crude protein concentrations ranging from 43.4 to 63.5% and 17.1 to 30.8%, respectively. Similar preparations from 21 selected *Arachis hypogaea* L. cultivars had 44.8 to 57.4% and 20.6 to 29.1% protein. Percentage oil in seed meals of wild species ranged between 46.5 and 63.1% while those of cultivars were 43.6 to 55.5%. Further characterization of proteins on polyacrylamide disc electrophoretic gels showed that most of the arachin and nonarachin components remained constant among cultivars while many new and variable patterns of these constituents were noted among wild *Arachis*. The gel data corroborated the known classification of wild *Arachis* into seven coherent sections; cultivated varieties could also be classified into seven groups. Quantities of protein and oil were randomly distributed among wild species and cultivars and could not be used to develop a chemotaxonomic relationship in *Arachis*.

Arachis hypogaea L. is a potential source of food-grade protein as it is readily cultivated commercially and has attained widespread acceptability because of its economic value to industry and dietary interest by the consumer. In addition, continued improvement of this commodity through genetic programs, updated agronomic practices, and modern food technology has helped to expand its food use. For example, research has shown that peanut seeds have potential as a source of food-grade protein for fortification of food products, and, through industrially applicable techniques, for recovery of meals, flours, flakes, grits, concentrates, and isolates (Ziemba, 1975; Quinn et al., 1975; Khan et al., 1975; Ayres et al., 1974; Mattil, 1973; Rhee et al., 1972, 1973; Harris et al., 1972; Smith, 1971; Mitchell and Malphrus, 1968; Altschul, 1967; Arthur, 1953; Pominski et al., 1952). However, only limited progress has been made in expanding utilization of peanuts specifically as a source of high protein food and research results have been much less extensive than those on soybeans and cereals (Pulle and Ino, 1975; Fan and Sosulski, 1974; Bushuk, 1974; MacRitchie, 1973; Wolf and Cowan, 1971).

Other studies have suggested that peanut seeds may be useful sources of proteins (Cherry et al., 1974, 1975; Cherry, 1974; McKinney et al., 1973; Goldblatt, 1971). In addition, studies have shown that peanut seeds are composed of groups of proteins including arachin (major storage globulin) and conarachin or nonarachin components (Basha and Cherry, 1976; Shetty and Rao, 1974; Cherry et al., 1973; Cherry and Ory, 1973; Singh and Dieckert, 1973a,b; Dawson, 1968, 1971; Neucere, 1969). These fractions were also shown to differ in amino acid composition (Basha and Cherry, 1976; Singh and Dieckert, 1973a,b; Dawson, 1968, 1971; Neucere, 1969). Thus, these protein fractions may differ not only in nutritional value but in functional properties. Partitioning these components may lead to expanded utilization of peanuts as protein ingredients in new foods as well as other more conventional peanut products.

Research in protein quality has only recently been reemphasized in the exploration for new sources of utilizable protein. Among these sources are the approximately 50 to 70 known wild species collections of the genus *Arachis* (Gregory et al., 1973; Cherry, 1975; Neucere and Cherry, 1975). Wild species contain new sources of germ plasm which can be used to increase variability in the genetic base of cultivated varieties (Simpson and Haney, 1973; Miller, 1973; Kamra, 1971; Frankel and Bennett, 1970; Nelson, 1969). Once the cross-compatibility of

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